A RAPID METHOD FOR PREPARING ANTIGENS FROM NORMAL HEART MUSCLE

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Since the discoveries of Landsteiner,[1] Pòrges and Meier,[2] and others, that alcoholic extracts of normal organs can be used as antigens, methods of preparing and standardizing antigens have been the subject of considerable study. Indeed, antigens are probably the all-important factor in the Wassermann reaction and uniformity of preparation is therefore highly desired. Thus far all attempts to standardize these preparations have not been particularly successful. This is primarily due to: (*a*) the variations of the lipoid content of different tissues; (*b*) kinds of solvents; (*c*) methods of extraction, and (*d*) periods of extractions.

The first factor is of special interest, since a comparison of plain alcoholic extracts from human hearts obtained at necropsy have given us variations in their fixative power from 1 : 10 to 1 : 180, using 0.05 and 0.1 c c of each dilution and 0.05 and 0.1 c c of the syphilitic serums. Considering the second and third factors, it is obvious that different fat solvents will yield different extractives, and these extractives will contain more or less anticomplementary and hemolytic substances. Erlandsen[3] has shown that when finely divided heart muscle, dried in the air, is completely extracted with ether and then with alcohol, the first extract contains the monophosphatids while the subsequent alcohol extract contains the diaminophosphatids which were not free in the tissues but existed in the combined state. Recently Neymann and Gager[4] advocated the extraction of the heart tissues with ether before its ultimate extraction with alcohol. According to these authors, the primary extracts contain no substances that have any antigenic value except lecithin, and this substance comprises less than one quarter of the entire weight of the ether extract, and tho it has a fairly high

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[1] Wiener Klin. Woch., 1907, 20, p. 1565.
[2] Berl. Klin. Woch., 1908, 45, p. 731.
[3] Zeitschr. f. Physiol. Chem., 1907, 51, p. 71.
[4] Journ. of Immunology, 1917, 2, p. 573.

binding power it is anticomplementary 1:20. The other extracts contain large amounts of fat and fatty acids, substances which are hemolytic in their properties.

Our series of extractions are comparisons between direct alcoholic and primary ether extractions followed by alcohol. As regards methods, the extractions made in the electrical shaker will certainly be stronger than those made by shaking the powder at varying intervals for the same unit of time. Finally, the period of extraction not only will influence the amount of antigenic substances, but also the amount of other extractives. We have further compared the extracts obtained in boiling alcohol, 78 C., with those obtained by an electrical shaker and those shaken at different intervals for different lengths of time at 37 C. With the exception of Landsteiner, Müller and Pötzl,[1] who extracted guinea-pig hearts with alcohol for about 10-12 hours at 60 C., the majority of investigators have applied lower temperatures during extraction.

TECHNIC

Three normal beef hearts were used. The pericardium, endocardium, larger blood vessels and fat were removed. The myocardium was finely ground and spread on glass plates and thoroughly dried at the incubator temperature. The dried material was then powdered and a total of 250 gm. obtained. The powder was divided in portions of 25 gm. and 75 c c of 95% ethyl alcohol added to each of five portions. The remaining five were extracted three times with ether using 50 c c per portion, each time for a period of 10 minutes. These portions were allowed to dry and then covered with 75 c c of alcohol. Two portions (one of ether and one of nonether extract) were extracted in a reflux condensor in the waterbath for 1 hour and two other portions for 3 hours. We have simply used a 40 inch glass tube of 3 mm. caliber as condensor. Two portions were extracted in an electrical shaker for 24 hours at room temperature; two for 2 days at 37 C., shaking 5 times a day, and the remaining two also 5 times a day for a period of 7 days. The extracts were carefully filtered and made up to 75 c c. In addition we compared these antigens with a good antigen which we have been using for many months. This antigen was treated with ether and extracted for 4 weeks with alcohol. the accompanying table gives the results of our titrations:

From these experiments it is evident that the boiled alcohol extractions are practically just as strong as those extracted under the usual conditions. The ether treated extractions have lost some binding power, especially the one boiled for 1 hour. The 24-hour shaken (ether extracted) has also lost considerable strength. All the others had more or less marked fixing power at as high a dilution as 1:200. The nonether extracted antigens contained throughout more anti-

TABLE 1

COMPARATIVE TITRATIONS OF ANTIGENS

Antigens 0.05 c c	1:10		1:50		1:100		1:150		1:200		No Serum Controls Antigens in Amounts of C C			
	Positive Serum	Negative Serum	Positive Serum	Negative Serum	Positive Serum	Negative Serum	Positive Serum	Negative Serum	Positive Serum	Negative Serum	0.005	0.01	0.03	0.05
1 hour boiled	++++	—	++++	—	+++	—	++	—	+	—	—	—	±	Inhibition
Ether extracted (boiled 1 hour)	++++	—	+++	—	++	—	+	—	—	—	—	—	—	Inhibition
3 hours boiled	++++	—	++++	—	++++	—	+++	—	+	—	—	—	+	Inhibition
Ether extracted (boiled 3 hours)	++++	—	++++	—	+++	—	++	—	±	—	—	—	—	Inhibition
24 hours shaken	++++	—	++++	—	+++	—	++	—	±	—	—	—	+	Inhibition
Ether extracted (shaken 24 24 hours)	++++	—	++++	—	++	—	+	—	—	—	—	—	—	Inhibition
2 days at 37 C.	++++	—	++++	—	+++	—	++	—	+	—	—	—	+	Inhibition
Ether extracted (2 days at 37 C.)	++++	—	++++	—	+++	—	++	—	±	—	—	—	—	Inhibition
7 days at 37 C.	++++	—	++++	—	++++	—	+++	—	+	—	—	—	+	Inhibition
Ether extracted (7 days at 37 C.)	++++	—	++++	—	++++	—	++++	—	+	—	—	—	—	Inhibition
Ether extracted (4 weeks at 37 C.)	++++	—	++++	—	++++	—	++	—	++	—	—	—	—	Inhibition

complementary substances showing slight inhibition of hemolysis when used in 0.3 c c quantities from a 1 : 10 dilution, while all inhibited hemolysis when used undiluted.

All the antigens were tested in order to determine their hemolytic properties, and it was found that no hemolysis took place when using 0.3 c c of a 1 : 10 dilution of the antigens. When these tubes are allowed to stand at room temperature for 3 hours or more hemolysis occurs in the 0.3 c c tubes. All these extracts can be safely used in dilutions of 1 : 20 and amounts of 0.05 and 0.1 c c giving excellent reactions.

CONCLUSIONS

Suitable antigens can be made by extracting normal heart tissues for a period from 1-3 hours with boiling alcohol in a reflux condensor.

The value of these antigens compares favorably with those extracted by the usual methods.

A primary extraction of the dried tissues with ether results in a slight loss of their fixing power, but yields extracts containing less anticomplementary substances.